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# THE NITROGEN OF PROCESSED FERTILIZERS.

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## INTRODUCTION.

Organic compounds have lately taken on a deeper significance in their relation to the complex problems of the soil and of crop production, for not only do they affect the physical conditions and chemical reactions of the soil but they also have been shown to be directly connected with fertility or infertility, some of them being essentially beneficial to the growth of plants, while others are distinctly harmful. Of the organic compounds thus far isolated from soils, a large number contain nitrogen, and of these nitrogenous substances, some have been found rather widely distributed in soils varying as to location, climate, methods of cropping, etc. These nitrogenous compounds occur either as plant constituents or arise from the decomposition of plant or animal protein, brought about by the various biological and biochemical agents in the soil. Not only compounds of this class found in soils but also many other protein decomposition products have been studied, both alone and in conjunction with the three fertilizer elements, in respect to their action on plant growth, and they have been shown in a number of cases to exert a beneficial influence; furthermore, these complex compounds are available for use by the plant without first being changed by chemical or biochemical means into ammonia and then to nitrates.[1]

That these facts have an immense practical bearing on fertilizers and the fertilizer industry, both from the standpoint of the producer and of the consumer, is at once obvious. The old high-grade nitrog-

[1] A Beneficial Organic Constituent of Soils: Creatinine. By Oswald Schreiner, E. C. Shorey, M. X. Sullivan, and J. J. Skinner. Bul. 83, Bur. Soils, U. S. Dept. Agr., 1911.
Nitrogenous Soil Constituents and Their Bearing on Soil Fertility. By Oswald Schreiner and J. J. Skinner, Bul. 87, Bureau of Soils, U. S. Dept. Agr., 1912.

This investigation is a contribution to the knowledge of the nature of the changes brought about in the manufacture of some of the processed fertilizers, and of the character and availability of such processed goods in mixed fertilizers when used in farm practice.

enous fertilizers, such as cottonseed meal, dried blood, fish scrap, etc., are being used more and more for feed purposes, and the time can not be far distant when their use as fertilizers will cease to be economic; thus a necessity for other and cheaper fertilizers of this type arises. Coupled with this is the desire of the chemist and the manufacturer to utilize in one way or another all waste products, whatsoever their nature, so that the number and kinds of nitrogenous materials which are used in the manufacture of fertilizers is on the increase. Described in the patent literature and found on the market are a large number of fertilizers which may be characterized as "processed," that is, the crude materials, not in themselves permissible as fertilizers, are made to undergo some decided chemical change to render them suitable as plant nutrients. It has been found that the "availability" of the crude substances is nearly always greatly increased by such processing and that a much larger percentage of the nitrogen in the finished product is soluble in water, although the actual chemical changes produced seem to have received little attention. The chemical compounds in processed fertilizers which are here shown to have direct fertilizer significance have not been determined, other than to show that ammonia is formed during processing and that ammonia is more readily produced from the processed goods.

Since the wastes from which this type of fertilizer is made contain more or less protein, or proteinlike substances, it seemed quite obvious that the finished fertilizers must contain more or less of the chemical compounds which would arise by such treatment from pure proteins in the laboratory. Since the action on plants of many of this class of compounds has been determined it is evident that the finding of such compounds in the fertilizers would throw much light on the question of the "availability" of the nitrogen in the fertilizer itself.

### BASE GOODS A TYPE OF PROCESSED FERTILIZER.

For a chemical study of processed fertilizers a sample of "wet-mixed" or "base goods" fertilizer was chosen as a representative of this type of fertilizer material. The base goods was obtained directly from the factory for use in this investigation. This fertilizer is made by the treatment of various trade wastes and refuse, such as hair, garbage tankage, leather scraps, etc., with rock phosphate and the requisite amount of sulphuric acid. These materials are mixed together in a "den" and the resulting mass is allowed to stand for several days, until it is cool enough to be conveniently handled. In the course of the reaction the mass reaches a temperature approximating 100° C., and the identity of the original substances is almost or entirely lost. Under these conditions it is certain that more or less

hydrolysis of the proteins in the crude materials takes place, with the formation of proteoses, peptones, polypeptides, or the simple amino acids, the kinds and number of products formed necessarily depending on the proportion of the different proteins in the original materials, on the amount and strength of the acid, the length of time of the reaction, and the temperature reached during the treatment.

Hartwell and Pember [1] have recently made a study of base goods in order to determine the availability of the nitrogen contained in it as compared with that of the high-grade nitrogenous fertilizers. The product which they used was made from hair tankage, garbage tankage, and roasted leather, together with rock phosphate and sulphuric acid. From their report the following figures for the analysis of the crude materials used in producing the fertilizer and of the finished product are taken·

TABLE I.—*Total nitrogen in cruae materials and finished product. (Hartwell and Pember.)*

| | Nitrogen. |
|---|---|
| | *Per cent.* |
| Hair tankage | 6.28 |
| Roasted leather | 6.49 |
| Garbage tankage | 2.87 |
| Base goods, including the above | 1.68 |
| Water soluble nitrogen in base goods | 1.28 |
| Water insoluble nitrogen in base goods | .40 |

TABLE II.—*Percentage of the total nitrogen present in different forms. (Hartwell and Pember.)*

| | Before putting into the den. | After removing from the den. |
|---|---|---|
| In ammonia | 6.5 | 14.3 |
| In water soluble organic matter | 7.8 | 57.7 |
| In water insoluble organic matter | 85.7 | 28.0 |

The experimental work of the present investigation was along two separate lines: (1) Analytical, involving total nitrogen determinations and the separate estimation of the various forms in which nitrogen may occur; (2) a determination of the definite chemical compounds present in the fertilizer by suitable methods of isolation and identification.

## THE CHEMICAL EXAMINATION OF BASE GOODS.

### TOTAL NITROGEN AND AMMONIA.

*Total nitrogen.*—The total in the base goods was determined by the Kjeldahl-Gunning-Arnold [2] method and was found to be 1.61 per cent.

[1] J. Ind. Eng. Chem., **4**, 441 (1912).

[2] U. S. Dept. Agr., Bureau of Chemistry, Circ., **108**, 15 (1912); T. C. Trescot, J. Ind. Eng. Chem., **5**, 914 (1913).

*Ammonia.*—Considerable difficulty was experienced in obtaining concordant results in the determination of the nitrogen in the form of ammonium salts. Boiling weighed amounts of the base goods with water and magnesium hydroxide, according to the official method,[1] for the determination of ammonia in fertilizers, did not give duplicate results sufficiently close for the purpose of this research. Owing to the acidity of the sample, it was impractical to use barium carbonate, but litharge was used with varying results. Finally, the determination was made by using the vacuum distillation method, which gave concordant results. This method, which gives only the nitrogen found as ammonia or as ammonium salts, is used for the determination of amide nitrogen in the products of acid hydrolysis of proteins. A weighed quantity of the fertilizer was placed in a Claisen flask connected up with a cooled receiver of 1 liter capacity and a small guard flask of 200 cubic centimeters capacity. Both flasks contained 0.1 N sulphuric acid. To the fertilizer was added 100 c. c. of neutral 95 per cent alcohol and 100 c. c. of distilled water, together with enough 10 per cent suspension of calcium hydroxide to make the mixture decidedly alkaline in reaction. The ammonia was then distilled under a pressure of from 10 to 12 mm., the temperature of the bath not exceeding 40° C. In the table which follows are given the results obtained by the three methods here used for the determination of ammonia.

TABLE III.—*Nitrogen in the form of ammonia or ammonium salts.*

| Method. | Expressed in per cent of base goods. | Expressed in per cent of total nitrogen in base goods. |
|---|---|---|
| Magnesium hydroxide distillation | 0.380 | 23.60 |
| | .389 | 24.16 |
| Lead oxide distillation | .394 | 24.47 |
| | .420 | 26.09 |
| Vacuum distillation | .374 | 23.23 |
| | .374 | 23.23 |

An examination of these results shows that by boiling with magnesia or litharge, somewhat more nitrogen is found as ammonia than really exists in this form in the base goods. It is therefore probable, that there are in the base goods nitrogenous compounds which are broken down into ammonia by the action of these alkaline reagents at a temperature of 100° C. The use of magnesia at boiling temperature for the purpose of determining the amount of ammonia split off by acid hydrolysis from certain proteins which contained cystine, was found to give unreliable results.[2] The reason for this

[1] Bul. 107, 9 (Revised), Bureau of Chem., U. S. Dept. Agr.

[2] Embden, quoted by Gümbel, Hofmeister's Beiträge, **5**, 297 (1904): Hart, Zeit. physiol. Chem., **33**, 354, 1901); Folin, ibid., **39**, 476 (1903); Denis, J. Biol. Chem., **8**, 427 (1910).

was found to be that magnesia under such conditions changes a part of the amino nitrogen of cystine into ammonia. In this laboratory it was also found that by boiling cystine with lead oxide one of the amino nitrogen groups of this compound was split off almost quantitatively, with the concurrent splitting off of hydrogen sulphide. Furthermore, it has been shown that if the amide nitrogen from protein hydrolysis is determined by distillation with a weak alkali, such as calcium hydroxide, at a temperature not to exceed 40° to 42° C. in the bath and at a pressure of from 10 to 12 millimeters, no decomposition of cystine takes place.[1]

In the manufacture of base goods the hair which is used contains proteins which on acid hydrolysis yield a high percentage of cystine. This fact, together with the analytical results just discussed, suggest rather strongly that there is present in the base goods more or less cystine, although this evidence can not be considered conclusive, since it is possible that in such a heterogeneous mixture there may be present other nitrogenous compounds which would be decomposed by magnesia or litharge with the liberation of ammonia.

## NITROGEN PARTITION.

For the purpose of determining the different forms of nitrogen present in the base goods the method of Van Slyke[2] was followed in its essential details, except that the determination of cystine, was not made. The method for the determination of this compound, according to the procedure used by Van Slyke, depends not upon a nitrogen determination but upon the determination of the amount of sulphur in the compounds precipitated by phosphotungstic acid. This determination when made on the hydrolytic products of acid digestion of pure protein may give quite satisfactory results, but the raw materials from which base goods are made contain many organic compounds other than proteins or protein decomposition products, and this is of course particularly true in the case of garbage tankage. It is well known that many plant and animal substances contain sulphur in a variety of linkages, and garbage tankage no doubt contains sulphur in other forms than that of cystine. The hair and leather used have both undergone some decomposition before the acid treatment and it is not impossible that the cystine originally present in the proteins may have been changed into sulphur compounds of a different chemical nature. No doubt some sulphur compounds other than cystine are precipitated by phosphotungstic acid, so that a determination of cystine depending on the sulphur content of the phosphotungstic acid precipitate would be of uncertain value in dealing with material of unknown origin and of such a heterogeneous character as fertilizer goods.

[1] Gümbel, Hofmeister's Beiträge, 5, 297 (1904). [2] J. Biol. Chem., 10, 15–55 (1911).

It should also be stated that although the results from the Van Slyke analysis are expressed in the usual way, arginine N, histidine N, etc., that it is not intended to convey the impression that these fractions contain pure arginine, histidine, etc., since as will be shown later, other compounds are included under these analytical terms. However, the nitrogen so expressed is that which is contained in compounds which give the various reactions upon which the Van Slyke method depends.

Two 20-gram samples of base goods were extracted for analysis. The first sample was extracted with boiling water until the extract ceased to give an acid reaction. The second sample was boiled for 24 hours with hydrochloric acid, sp. gr. 1.115, the resulting solution was filtered by suction and the insoluble residue washed with hot water until the washings ran free from chlorides. The two extracts were then concentrated to the consistency of a sirup in vacuo to expel the free volatile acid, and each was finally made up to a volume of 250 c. c.

*Total nitrogen.*—Total nitrogen in solution was determined by subjecting 50 c. c. of the solution to Kjeldahl analysis. The water extract contained 1.372 per cent and the hydrochloric-acid extract 1.435 per cent of the base goods.

*Amide nitrogen.*—Amide nitrogen was determined by distilling in vacuo the remaining 200 c. c. of solution, to which were added 100 c. c. of 95 per cent alcohol and 20 c. c. of a 10 per cent suspension of calcium hydroxide, as described under the determination of ammonia. The water extract contained 0.374 per cent and the hydrochloric acid extract 0.882 per cent.

*Humin nitrogen.*—The residue from the amide nitrogen determination was used for the determination of humin nitrogen. The precipitate, formed by the addition of calcium hydroxide, was filtered off and washed with distilled water in the same manner in which Van Slyke directs that the phosphotungstic acid precipitate be washed. The washing was continued until no reaction for chlorides or alkalinity was obtained. The nitrogen remaining in the precipitate and in the filter paper was then determined by Kjeldahl analysis. The humin nitrogen was 0.031 per cent for the water extract and 0.074 per cent for the hydrochloric acid extract.

*Diamino acid nitrogen.*—The combined filtrate and washings from the humin precipitate were neutralized with hydrochloric acid, concentrated in vacuo to a volume of about 100 c. c. and then transferred to a 300 c. c. Erlenmeyer flask. To this solution were added 18 c. c. of concentrated hydrochloric acid together with 15 grams of purified phosphotungstic acid [1] and the whole diluted with water to a volume of 200 c. c. The flask was placed on a steam bath and heated until

[1] Winterstein, Zeit. physiol. Chem., 34, 153 (1901).

the phosphotungstates were almost redissolved, when it was set aside for 48 hours in order to allow them to recrystallize and fully precipitate. The precipitate was then filtered, washed, and dissolved in 45 per cent sodium hydroxide as described by Van Slyke. The phosphotungstic acid was precipitated with barium chloride and filtered off. The filtrate and washings from this precipitate were concentrated in vacuo and made up to a volume of 200 c. c.

*Arginine nitrogen.*—Arginine nitrogen was determined in 100 c. c. of this solution by boiling with 12.5 grams of solid potassium hydroxide for six hours and collecting the ammonia formed in 0.1 N sulphuric acid. Under these conditions one-half of the nitrogen in the arginine and 18 per cent of the nitrogen of cystine is split off as ammonia.

*Total nitrogen in the diamino acid solution.*—Total nitrogen in the diamino acid solution was found by subjecting the solution remaining after the arginine determination to Kjeldahl analysis and adding to the ammonia so obtained the amount obtained from the arginine nitrogen determination.

*Amino nitrogen.*—Amino nitrogen was determined by means of the Van Slyke apparatus.[1]

From these three figures the nitrogen was calculated as arginine N, histidine N, and lysine N according to the two formulas:

(1) Histidine N = 1.667 non-amino N − 1.125 arginine N;

(2) Lysine N = total N − (arginine N + histidine N).

The results obtained were as follows: For the water extract arginine 0.111 per cent, histidine nitrogen 0.117 per cent, and lysine nitrogen 0.081 per cent; for the hydrochloric-acid extract they were 0.104, 0.070, and 0.117 per cent, respectively.

*Total nitrogen of the monoamino acids.*—To the combined filtrate and washings from the phosphotungstic acid precipitate 45 per cent caustic soda was added until the solution became turbid by the precipitation of lime; acetic acid was then added until the solution cleared. This solution was placed in a 500 c. c. flask and made up to the mark. Total nitrogen was estimated in 100 c. c. portions, using the Kjeldahl method.

*Amino nitrogen.*—Amino nitrogen in the form of monoamino acids was determined by use of the Van Slyke apparatus.

From the two figures obtained the amount of nitrogen present as *non-amino nitrogen* in monoamino acids was found by difference. The amino nitrogen in the form of monoamino acids in the water extract was 0.543 per cent and in the hydrochloric acid extract 0.546 per cent. The non-amino nitrogen in the monoamino acid fraction of the water extract was 0.114 per cent and in the hydrochloric acid extract it was 0.133 per cent.

[1] For the description of this apparatus and the details of the procedure employed, see: Van Slyke, Jour. Biol. Chem., 12, 275 (1912).

Van Slyke has shown that certain corrections must be applied in the method, owing to the fact that the phosphotungstates of the diamino acids are slightly soluble, and these corrections have been applied just as though the fractions contained only the hydrolysis products of pure proteins. In Table V the combined results of the analyses are given.

The above analytical procedure which separates the nitrogen into different groups, gives results than can only be rigidly interpreted when the products of the acid hydrolysis are known. The results of the analysis of base goods by this method can only be clearly understood when further facts regarding the compounds, in which the nitrogen is contained, are discovered. A description of the methods used in isolating and identifying certain of these compounds follows.

## ISOLATION AND IDENTIFICATION OF DEFINITE COMPOUNDS FROM THE PROCESSED FERTILIZER.

Ten pounds of base goods were extracted by boiling for 1 hour with 20 gallons of water in a steam-jacketed kettle. The solution was filtered from the insoluble residue, made exactly neutral with caustic soda, the precipitate formed filtered off, and the filtrate concentrated in a steam kettle to a volume of about 3,500 c. c.

This solution contained phosphates, sulphates, and much other mineral matter. In order to separate as much of these salts as possible from the organic compounds a cold saturated solution of barium hydroxide was added to the solution until no further precipitation took place. The heavy precipitate which formed was filtered off by suction and washed many times with water. The filtrate was exactly neutralized with sulphuric acid and concentrated to a volume of about 2,000 c. c. After cooling, this solution was made acid to 5 per cent with sulphuric acid and a solution of phosphotungstic acid was added to slight excess, and the mixture allowed to stand.

After 3 days the precipitate which formed was filtered off and washed with water containing about 5 per cent sulphuric acid and a little phosphotungstic acid. The precipitate was carefully dissolved in 45 per cent caustic-soda solution, using phenolphthalein as an indicator and adding at no time more than two drops of the alkali solution. Water was added so that a volume of about 1,500 c. c. was reached, and barium hydroxide solution was added until the phosphotungstic acid was precipitated. After filtering off the barium phosphotungstate, the free alkali was just neutralized with sulphuric acid, and the solution was then evaporated almost to dryness with barium carbonate in order to expel all of the ammonia. The residue was taken up in about 1,000 c. c. of hot water and filtered, and the precipitate washed with hot water. The filtrate was placed in a 5-liter flask and treated while hot with solid silver sulphate, which was added slowly until the

solution contained sufficient to give a yellow precipitate, when a drop was removed and tested with a solution of barium hydroxide. The solution was then filtered, and the separation of the three hexone bases was carried out according to the method of Kossel and Kutscher.[a] The solution was cooled to 40° C. and saturated with finely powdered barium hydroxide. The precipitate which was formed was collected and stirred up in a mortar with solid barium hydroxide, when it was again filtered off and washed with barium-hydroxide solution. This precipitate contains the silver salts of histidine and arginine, while the filtrate contains the lysine.

*Lysine.*—The above filtrate was acidified with sulphuric acid and freed from silver with hydrogen sulphide. Lysine was precipitated from this solution as the phosphotungstate, and the free base was obtained by decomposing this salt with barium hydroxide. From a concentrated solution of the base, which was strongly alkaline in reaction and which showed no tendency to crystallize on standing, the picrate salt was prepared. This compound showed the solubility, characteristic crystalline appearance, and properties of lysine picrate.[b] When taken up in boiling water and allowed to crystallize slowly, it formed in rather large yellow prisms, but when in small amount the crystals assumed a fernlike appearance. The lysine was further identified by the preparation from the picrate of the hydrochloride salt, $C_6H_{14}O_2N_2.2\ HCl$, and the platinum chloride salt, $C_6H_{14}O_2N_2.H_2Pt\ Cl_6 + C_2H_5\ OH$.[c]

The silver precipitate which would contain the arginine and histidine was suspended in water acidified with dilute sulphuric acid and broken up with hydrogen sulphide. The silver sulphide was filtered off, the sulphuric acid was removed with barium hydroxide solution, and after filtering the solution was made slightly acid with nitric acid. Silver nitrate solution was added until a test drop with barium hydroxide gave a yellow precipitate. Histidine was completely precipitated as the silver salt by the careful addition of barium hydroxide solution. The precipitate was washed with barium hydroxide solution until the washings ceased to give a test for nitrates.

*Histidine.*—The histidine silver was suspended in water acidulated with sulphuric acid and treated with hydrogen sulphide. The procedure described by Kossel and Kutscher was followed, and the histidine was finally separated as the dihydrochloride salt. The method of obtaining this compound and the characteristic crystalline form of the dihydrochloride salt[d] are sufficient to establish its identity as histidine.

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[a] Zeit. physiol. Chem., **31**, 166 (1900).
[b] Kossel, Zeit. physiol. Chem., **25**, 180 (1898); **26**, 586 (1899).
[c] Hedin, Zeit. physiol. Chem., **21**, 299 (1895).
[d] Schwantke, Zeit. physiol. Chem., **29**, 492 (1900); Kossel, ibid., **22**, 182 (1896).

*Arginine.*—The method of isolating arginine is simply a further step in the method used in the isolation of histidine. Arginine was isolated first as the acid nitrate salt, which crystallized in the form of plates,[1] and was further identified by preparing the neutral nitrate salt and the copper nitrate salt both in characteristic crystalline form.

*Monoamino acids.*—The filtrate from the phosphotungstic acid precipitate was made alkaline with barium hydroxide in order to remove the sulphuric and phosphotungstic acids, and filtered. The filtrate was concentrated and nearly neutralized with sulphuric acid. This slightly alkaline solution, about 500 c.c. in volume, was treated by boiling with freshly prepared copper hydroxide, and was then poured into about 3,000 c.c. of 95 per cent alcohol and allowed to stand over night, in order that the insoluble mineral matter might settle out. The deep-blue alcoholic solution was then filtered, the insoluble salts redissolved in water, and reprecipitated by pouring into alcohol as before. The alcoholic solutions were combined and evaporated to dryness, the residue was taken up in hot water and the copper removed by treatment with hydrogen sulphide. After filtering from the copper sulphide, the solution, which contained considerable color, was boiled with animal charcoal. The filtered solution was made faintly alkaline with ammonia and treated with freshly precipitated copper hydroxide, keeping the volume of the solution at about 1,000 c.c. The solution was filtered from the excess of copper hydroxide and evaporated to dryness on the steam bath. The solid residue was then scraped from the sides of the dish and extracted in a Soxhlet extractor with absolute methyl alcohol until no further blue color was imparted to the alcohol.

*Leucine.*—The alcohol insoluble portion was dissolved in a large volume of boiling water and the copper removed with hydrogen sulphide. The solution was filtered, boiled down to a volume of about 50 c.c. and treated with ammoniacal lead acetate until no further precipitation took place. The precipitate was washed with 95 per cent alcohol and was finally decomposed with hydrogen sulphide after suspending in water. On concentration of a portion of this solution the characteristic crystals of impure leucine formed. These crystals separated in concentric nodules closely resembling fat, but which were composed of concentrically grouped highly refracting needles. These crystals were redissolved in water and added to the original solution which was boiled up with animal charcoal until the color disappeared. The leucine was then purified as before by the formation of the copper salt and the basic lead salt. On concentrating the solution obtained from this purification, crystals of pure leucine were obtained. These crystals formed in pearly scales, which somewhat

[1] See Gulewitsch, Zeit. physiol. Chem., 27, 178 (1899).

resemble cholesterin. When dry the crystals were light, had a satiny glossy appearance, and were not easily wet again with water, They were extremely soluble in hot water and quite easily soluble in cold water. Leucine was further identified by the fact that it sublimed,[1] and by the crystalline form and solubility of the copper salt,[2] and by its two color reactions with quinone,[3] red with a solution of leucine and quinone and violet when in addition sodium carbonate was used.

*Tyrosine.*—The methyl alcohol solution of the copper salts was evaporated to dryness, and the residue taken up in water. The copper was removed with hydrogen sulphide and the solution was boiled with animal charcoal. After filtering, the solution was concentrated and long thin silky needles began to separate. These needles, which closely resembled tyrosine, were filtered off, and the filtrate further concentrated, when another crop of needles was obtained. These were filtered off and added to the first fraction and were then extracted with boiling 70 per cent alcohol. The crystalline residue was recrystallized from water a number of times and dried on a porous plate. This compound crystallized in the stellate groups of long slender silky needles which are characteristic of tyrosine. These crystals were relatively insoluble in cold water,[4] very insoluble in cold 90 per cent alcohol, easily soluble in hot water, and were tasteless, colorless, and infusible. The compound was further identified as tyrosine by the formation of the copper salt, which was rather insoluble in cold water and fairly easily soluble in hot water, by the fact that a solution of the compound gave a red color when boiled with Millon's reagent,[5] and that a sulphonic acid prepared from the compound gave a violet color with ferric chloride.[6]

*Purine bases.*—Five pounds of base goods were boiled up with 10 liters of water, filtered, neutralized and concentrated to a volume of about 2,500 c. c. The solution was made strongly alkaline with sodium hydroxide and the purine bases were precipitated with Fehling's solution and dextrose according to the method of Balke.[7] The supernatant liquid was decanted from the copper precipitate and this was washed, until free from alkali, with a solution of sodium acetate, by repeated decantations. The precipitate was filtered, freed from sodium acetate by washing with alcohol, and the copper removed by suspending the precipitate in water and treating it with hydrogen sulphide. After filtering off the copper sulphide the solution was concentrated and the purine bases reprecipitated by means

[1] Schwanert, Liebig's Ann., **102**, 224 (1857).
[2] Hofmeister, Liebig's Ann., **189**, 16 (1877).
[3] Wurster, Centrlb. Physiol., **2**, 590 (1889).
[4] Erlenmeyer and Lipp., Liebig's Ann., **219**, 161 (1883).
[5] Millon, Compt. rend., **28**, 40 (1849); Lassaigne, Ann. Chem. Phys. (2) **45**, 435 (1830).
[6] Piria Liebig's Ann., **82**, 252 (1852).
[7] Jour. prakt. Chem. [2], **47**, 537 (1893).

of a solution of silver nitrate and ammonia. After washing with water the silver precipitate was boiled with 10 c. c. of nitric acid, specific gravity 1.1, and filtered. From this solution, on cooling and standing, crystals were deposited which were filtered off.

The filtrate was diluted with water, made alkaline by the addition of ammonia, and a solution of silver nitrate added. No precipitate was formed showing the absence of xanthine.

*Guanine.*—The precipitate from the nitric acid solution was washed with water, suspended in water, and decomposed with hydrogen sulphide. The solution was filtered and concentrated to about 10 c. c. when strong ammonia was added producing a white gelatinous precipitate which was filtered off and washed with a little cold water. The precipitate was dissolved in a little warm hydrochloric acid and tested for the presence of guanine by means of the xanthine reaction and Weidel's test, both of which were positive. From the remainder of the solution the characteristic picrate of guanine described by Capranica [1] and the dicromate described by Wulff [2] were prepared. The method of obtaining this base, its solubility in water, ammonium hydroxide, and hydrochloric acid, the solubility of the silver salt in nitric acid, specific gravity 1.1, the color reactions, and the formation of the two characteristic salts, the picrate and dichromate, are sufficient to establish the identity of the compound as guanine.

*Hypoxanthine.*—The filtrate from the ammonia precipitation of guanine was boiled to expel all the ammonia and to a portion of the solution a solution of picric acid was added, but no precipitate was immediately formed, showing the absence of adenine. To another portion of the solution hydrochloric acid was added and the solution was concentrated when crystals resembling those of hypoxanthine hydrochloric separated out in whetstonelike crystals or bunches of prisms. Hypoxanthine forms a characteristic silver nitrate salt [3] and a characteristic silver picrate salt [4] both of which are crystalline and relatively insoluble in water. Hypoxanthine does not give the xanthine reaction, but when treated with nitric acid and bromine water a yellow color is produced which on addition of sodium hydroxide turns red, and on heating acts like the xanthine reaction. By means of these reactions the substance was identified as hypoxanthine.

### THE CHEMICAL CHANGES INVOLVED IN PROCESSING.

The compounds which were isolated from the base goods are tabulated in Table IV according to the sources from which they have been derived and the chemical groups to which they belong. While it was not possible to isolate these compounds in a strictly quantitative manner, nevertheless it was evident that the purine bases were

[1] Zeit. physiol. Chem., **4**, 233 (1880).
[2] Ibid., **17**, 477 (1893).
[3] Neubauer, Zeit. analyt. Chem., **6**, 34 (1867).
[4] Bruns, Zeit. physiol. Chem., **14**, 555 (1890).

present in exceedingly small quantities, although the method used in their isolation was subject to no more error than some other of the isolation methods; this would indicate that the nitrogen of the purine bases makes up but a small percentage of the total nitrogen present in the fertilizer.

TABLE IV.—*Organic compounds isolated from sample of base goods.*

| Compound. | Chemical group. | Source of compound. |
|---|---|---|
| Arginine | Diamino acids or hexone bases. | Products of protein hydrolysis by acid treatment of raw materials. |
| Histidine | | |
| Lysine | | |
| Leucine | Monoamino acids. | |
| Tyrosine | | |
| Guanine | Purine base | Plant constituent, or product of hydrolysis of nucleoprotein. |
| Hypoxanthine | do | Plant constituent, or product of conversion of nucleoprotein-base. |

*Purine bases.*—It will be noticed that the two purine bases are listed in the table as coming from different sources. It is a well-known fact that the purine bases may exist in plant tissues and plant extracts as such; that is, they are not linked up in more complex compounds in such a way that their peculiar chemical identity is lost. In the garbage which has entered into the manufacture of the fertilizer there were doubtless many sorts of plants or plant remains which contained some or all of the purine bases, and this fact alone would account for the presence of hypoxanthine and guanine in the finished product. This, however, is not the only source of the purine bases. Levene [1] and his associates have demonstrated that some of the purines enter into the composition of the nucleic acids, which are decomposition products of nucleoprotein and that they may be obtained by a process of hydrolysis from these nucleic acids. Of the four purine bases commonly encountered, only guanine and adenine have been found to be constituent parts of the nucleic acid molecule, it matters not whether the nucleic acid be a decomposition product of animal or plant nucleoproteins. But it has been shown that the two purines found in the nucleic acids may be changed, both by chemical and biochemical agencies, into the two other purine bases, xanthine and hypoxanthine, so that these are frequently encountered. Thus by the treatment of guanine with nitrous acid Fischer [2] changed it into xanthine and in the same manner Kossel [3] changed adenine into hypoxanthine. Furthermore, Schittenhelm and Schröter [4] have shown that the putrifactive bacteria, especially the colon bacillus,

[1] Levene and Jacobs, Ber., **44**, 746 (1911); Biochem. Zeit., **28**, 127 (1910); Levene, Abderhalden's Biochem. Arbeitsm., II, 605 (1910); Ibid., V, 489 (1911).
[2] Liebig's Ann., **215**, 309 (1882).
[3] Zeit. physiol. Chem., **10**, 258 (1886).
[4] Zeit. physiol. Chem., **41**, 284 (1904).

were able to convert adenine and guanine into hypoxanthine and xanthine. They also show that the bacteria have the power of splitting the nucleic acid itself. This same change is also brought about by the action of certain enzymes, such as erepsin, on nucleic acid.

With these facts at hand it is possible to draw the following conclusions as to the source of the two purine bases in this fertilizer: The guanine and hypoxanthine may be derived from plant remains which originally contained these two compounds; the guanine may arise by the acid hydrolysis of certain vegetable or animal nucleoproteins which were present in the original materials; and the hypoxanthine may have been formed by the processes of natural decomposition, such as the action of bacteria and enzymes, which had taken place in the crude materials before they were subjected to the acidulation process or during the process itself. It is not improbable that the guanine and hypoxanthine come from all of these sources.

*Diamino acids.*—Of the three diamino acids lysine was obtained in much the largest amount, arginine next, and histidine in the smallest amount. These compounds are products of protein hydrolysis by acids, but may also be produced under certain conditions by the action of bacteria. Since one or more of the diamino acids have been found to be present in every protein so far examined, and since the method for the analysis and the isolation of these bases is almost quantitative, the determination of the number and amounts of the diamino acids present in a mixture of protein hydrolysis products is of importance in deciding the nature and character of the original material which entered into the processed goods.

*Monoamino acids.*—Although leucine and tyrosine, which are protein decomposition products, were found in about the same quantities, the methods of isolation were so far from being quantitative that this relationship is of no significance. The isolation and identification of the other monoamino acids from the complex products of protein hydrolysis can only be accomplished, in the majority of cases, by means of the esterification method of Emil Fischer. This method is not a strictly quantitative one and requires large amounts of materials for a successful separation, and consequently was not used in this investigation. The use of methods other than that of esterification failed to isolate any other monoamino acid in quantities large enough for identification. As will be shown later, a number of monoamino acids besides the two isolated must be present in the processed goods.

Establishing the presence of these products of acid hydrolysis of proteins, namely, the diamino acids, arginine, lysine, and histidine, and the two monoamino acids, leucine and tyrosine, in the amounts in which they were found is of itself sufficient evidence to demonstrate that by the acid treatment of the crude materials used in the manu-

facture of the base goods the proteins contained therein have been changed. This change is shown to be a deep-seated one, since five of the compounds which are known to be final products of protein hydrolysis by acids are found. This, however, can not be taken to mean that the proteins have been completely hydrolysed by the acid treatment since it is possible to have present in the product of partial hydrolysis of proteins not only the diamino and monoamino acids, but also such intermediate compounds as polypeptids, peptones, proteoses, etc.

In this connection the results obtained by use of the Van Slyke method, which are given in Table V, are of particular interest. As has been already stated, the base goods were extracted (1) with boiling water and (2) with boiling acid. In the former case only slight further hydrolysis of the materials in the base goods is to be expected since the free acid in the fertilizer is extremely weak, and the boiling temperature, 100° C., is that which was reached in the process of manufacture. In the case of the second extract complete hydrolysis of all the proteins or proteinlike materials is certainly to be expected, since in addition to the original hydrolysis the material was boiled with strong hydrochloric acid for 24 hours, which treatment in the case of most proteins is sufficient for complete hydrolysis. The differences in the results obtained from the analyses of the two extracts may, therefore, be expected to throw some light on the question of the completeness of hydrolysis of the original proteins by the acid processing.

TABLE V.—*Nitrogen forms as determined by the Van Slyke method.*

| Form of nitrogen. | Results expressed in per cent of base goods. | | Results expressed in per cent of total N in base goods. | |
|---|---|---|---|---|
| | $H_2O$ extract. | HCl extract. | $H_2O$ extract. | HCl extract. |
| Total N | 1.610 | 1.610 | | |
| Total soluble N | [1] 1.372 | 1.435 | [1] 85.24 | 88.64 |
| Total insoluble N | [1] .238 | [1] .175 | [1] 14.76 | [1] 11.36 |
| Amide N | .374 | .382 | 23.23 | 23.70 |
| Humin N | .031 | .074 | 1.95 | 4.61 |
| Diamino acid fraction: | | | | |
| Arginine N | .111 | .104 | 6.89 | 6.46 |
| Histidine N | .117 | .070 | 7.26 | 4.38 |
| Lysine N | .081 | .117 | 5.06 | 7.26 |
| Monoamino acid fraction: | | | | |
| Amino N | .543 | .546 | 33.75 | 33.92 |
| Nonamino N | .114 | .133 | 7.10 | 8.27 |

[1] Obtained indirectly.

First it will be noticed that total soluble nitrogen in the hydrochloric acid extract is 88.64 per cent of the total N, while that of the water extract is 85.24 per cent, showing a difference of 3.4 per cent soluble N produced by further hydrolysis of the materials in the base goods. Correspondingly there is a decrease of insoluble N.

There is an increase of 0.47 per cent amide N in the hydrochloric acid extract over that in the water extract. This is due to the splitting off of ammonia from some nitrogenous compounds by the hydrochloric acid and suggests the presence of some product of partial protein hydrolysis in the fertilizer which contains an acid amide linkage.

The statement has already been made that nitrogenous compounds other than arginine, histidine, and lysine are included under the figures given for these compounds in the table. This is due to the fact that the phosphotungstic acid which is used as a precipitant of the diamino acids also precipitates peptones, proteoses, etc., as well as the purine bases, cystine, and possibly other compounds. Since nitrogen compounds other than proteins existing in the original material and susceptible to decomposition with hot acid, would have been already broken up in the processing, it follows that the changes produced by further boiling with acid would result from peptones, proteoses, etc. The difference noted between the results obtained from the two extracts for the diamino acids are therefore due to some interferring substances of the nature of proteins and not to such substances as the purines or cystine. Moreover, the latter compounds will produce the same relative error in analysis in the case of both extracts.

Of the diamino acids the only one determined directly is arginine. Its determination depends on the fact that when arginine is boiled for some time with strong potassium hydroxide, half of the nitrogen of the arginine is split off as ammonia. However, if cystine is present 18 per cent of its nitrogen is evolved as ammonia, together with the arginine nitrogen. As has already been stated this figure should be the same for the two extracts providing that there is present in the base goods no substance precipitated by phosphotungstic acid, and giving off ammonia when boiled with strong alkali or strong hydrochloric acid. A comparison of the results obtained for arginine in the two extracts shows that the figure for arginine in the water extract is higher than that of the hydrochloric acid extract by 0.43. In other words, there appear to be present in the diamino acid fraction compounds which on boiling with alkali give off ammonia amounting to 0.22 per cent of the total nitrogen. These compounds are broken up by the further hydrolysis with acid.

Further information may be obtained by a consideration of the figures for lysine and histidine, which are obtained not by a direct determination, but by calculation from the figures obtained for arginine N, total N in the fraction, amino N and non-amino N. Lysine contains only amino N, histidine contains one-third amino N and two-thirds non-amino N, while arginine contains one-fourth amino N and three-fourths non-amino N. Since histidine N is in a measure

obtained by difference from the non-amino N and the arginine N according to formula (1) on page 7, it is evident that if there are precipitated by the phosphotungstic acid compounds which contain non-amino N other than arginine and histidine, such nitrogen will be classed as histidine N, because the arginine N is determined directly.

A comparison of the results for histidine shows that there is 2.88 per cent less N calculated as histidine in the hydrochloric acid extract than in the water extract and at the same time there is an increase in lysine N in the hydrochloric acid extract amounting to 2.20 per cent. This shows that by the hydrolysis with hydrochloric acid some substance which reacted as though it contained non-amino N has been decomposed with the formation of an almost corresponding amount of amino N. Here again the indications are that this substance is of the class of compounds related to the proteins.

This is further borne out by the fact that in the monoamino acid fraction the nitrogen listed as amino N has increased in per cent 0.17 and the nitrogen as non-amino N has increased in per cent 1.17 by hydrolysis with hydrochloric acid.

A comparison of the figures for humin N shows an increase of 2.66 in the hydrochloric acid extract, but since the nature of the compounds in which this class of nitrogen exists is not understood no interpretation can be given to this figure.

*Proteoses.*—In order to prove the presence of some intermediate product of protein hydrolysis, which is thus indicated by analytical methods, an aqueous solution of about 2.5 pounds of base goods was made and the diamino acids were precipitated with phosphotungstic acid, in the presence of 5 per cent sulphuric acid. The precipitate which formed was allowed to stand over night and after filtering off it was washed well with 5 per cent sulphuric acid. The precipitate was dissolved in sodium hydroxide, the phosphotungstic acid precipitated by adding barium hydroxide solution, and after filtering the excess of barium was removed by adding sulphuric acid until a neutral reaction was obtained. Portions of this solution were tested for peptones, proteoses, etc., with the following results; The biuret test was positive; a precipitate was obtained on saturation of the solution with ammonium sulphate, or with sodium chloride; when the filtrate from the latter solution was treated with acetic acid a cloudy precipitate developed. Precipitates were also obtained with sulphuric acid, hydrochloric acid, phosphomolybdic acid and with phosphotungstic acid. A precipitate was formed on the addition of alcohol to the solution. This precipitate was filtered off, dissolved in dilute alkali, and on addition of very dilute copper sulphate solution the biuret reaction was again obtained. These reactions are those which are given by proteoses and by the proteins and confirm the conclusions

arrived at from the results obtained with the Van Slyke method. The Millon reaction and the Hopkins-Cole reaction were both negative, showing the absence from this proteinlike compound of the tyrosine and the tryptophane radicles.

A very large number of compounds intermediary between the protein and its primary hydrolysis products may occur, depending on a great variety of conditions so that the actual identification of the compound under discussion would be a difficult matter. However, the nature of this compound may be approximately determined by the results obtained in the study of the two extracts by the Van Slyke method. These results have been already discussed and they indicate the presence in the base goods of a compound of a proteose nature, which because it gives a biuret test, must be composed of at least three amino acids. The results indicate still further that the compound is composed of acid amide radicals, diamino acids, particularly lysine, and monoamino acids, those containing amino nitrogen and especially those containing non-amino nitrogen. Since the figures obtained by the nitrogen partition method are subject to a certain amount of error when applied to such a mixture the figures can only be taken as approximate for the various forms of nitrogen which make up this compound.

The figures given for arginine in the table are probably only influenced by any cystine present. Attempts to isolate cystine from the base goods failed, although it seems unlikely that this compound can be absent. The figures for histidine and lysine are undoubtedly too high, since they include all of the other nitrogenous compounds precipitated by phosphotungstic acid, so that the absolute amount of these compounds in base goods can not be correctly determined by this method. The figure given for the amount of amino nitrogen present as monoamino acids may be a little high, while the non-amino nitrogen figure is open to considerable error.

In Table VI are given the primary hydrolysis products of a number of proteins which may be present in the base goods. These results were obtained by the esterification method and show how the different proteins vary in the nature and amount of the units composing them. Many monoamino acids, besides leucine and tyrosine, occur in these proteins, and there must consequently be present in the base goods amino acids other than the two isolated. This is apparent from the composition of the various proteins shown in the table. Owing to the large amount of amide nitrogen present in the fertilizer, which was split off by the acidulation of the original proteins of the trade wastes, it may be concluded that considerable quantities of aspartic or glutamic acids are present in this sample of base goods.

The conclusions which are to be drawn from the results obtained by the examination of this fertilizer by means of the analytical and

isolation methods are as follows: The process by which the nitrogen of certain trade wastes, such as hair, leather, garbage, etc., is made more available, is recognized as a process of partial hydrolysis of the complex protein contained in such materials, resulting in ammonia, amino acids, etc., all of which are more available than the original protein material. This hydrolysis is almost complete, the nitrogenous compounds formed being principally the primary products of protein hydrolysis, together with a small amount of proteoselike compound which has not been fully decomposed.

TABLE VI.—*Products of acid hydrolysis of various proteins.*

| Compound. | "Synotin" from cattle flesh.[1] | "Keratin" from sheep's horn.[2] | "Keratin" from sheep's wool.[3] | "Keratin" from horse's hair.[4] | Halibut muscle.[5] | Ox muscle.[6] | "Legumin" from pea.[7] |
|---|---|---|---|---|---|---|---|
| Glycine[8] | 0.5 | 0.8 | 0.6 | 4.7 | 0.0 | 2.1 | 0.4 |
| Alanine[8] | 4.0 | 1.6 | 4.4 | 1.5 | (?) | 3.7 | 2.1 |
| Valine | .9 | 4.5 | 2.8 | .9 | .8 | .8 | |
| Leucine[8] | 7.8 | 15.3 | 11.5 | 7.1 | 10.4 | 11.7 | 8.0 |
| Isoleucine | | | | | | | |
| Phenylalanine[8] | 2.5 | 1.9 | | .0 | 3.1 | 3.2 | 3.8 |
| Tyrosine[8] | 2.2 | 3.6 | 2.9 | 3.2 | 2.4 | 2.2 | 1.6 |
| Serine | | 1.1 | .1 | .6 | (?) | (?) | .5 |
| Cystine | | 7.5 | 7.3 | 8.0 | | | |
| Proline | 3.3 | 3.7 | 4.4 | 3.4 | 3.2 | 5.8 | 3.2 |
| Oxyproline | | | | | | | |
| Aspartic acid[8] | .5 | 2.5 | 2.3 | .3 | 2.8 | 4.52 | 5.3 |
| Glutamic acid[8] | 13.6 | 17.2 | 12.9 | 3.7 | 10.1 | 15.5 | 17.0 |
| Tryptophane | | | | | (+) | (+) | (+) |
| Arginine[8] | 5.1 | 2.7 | | 4.5 | 6.4 | 7.5 | 11.7 |
| Lysine[8] | 3.3 | .2 | | 1.1 | 7.5 | 7.6 | 5.0 |
| Histidine[8] | 2.7 | | | .6 | 2.6 | 1.8 | 1.7 |
| Ammonia[8] | .9 | | | | 1.4 | 1.1 | 2.1 |
| Total | 47.3 | 62.3 | 49.2 | 39.6 | 50.7 | 67.5 | 62.4 |

[1] E. Abderhalden and T. Saski, Zeit. physiol. Chem., **51**, 404 (1907).
[2, 3] E. Abderhalden and A. Voitinovici, ibid., **52**, 348 (1907).
[4] E. Abderhalden and H. G. Wells, ibid., **46**, 31 (1905); A. Argiris, ibid., **54**, 86 (1905).
[5] T. B. Osborne and F. W. Heyl, Amer. J. Physiol., **22**, 433 (1908).
[6] T. B. Osborne and D. B. Jones, ibid., **24**, 437 (1909).
[7] T. B. Osborne and F. W. Heyl, J. Biol. Chem., **5**, 197 (1908).
[8] Physiological action on plant growth has been determined and reported in Bul. 87, Bureau of Soils, U. S. Dept. Agr.

## AVAILABILITY OF THE NITROGEN OF ORGANIC FERTILIZERS.

The question of the availability of the different kind of nitrogen contained in organic fertilizers is one that has caused considerable discussion. A number of methods have been proposed for determining this factor, and while some of them give helpful results, all excepting the plant method are open to more or less objection. The reason for this is that the methods are empirical and the nature of the complicated compounds in which the nitrogen is linked in the fertilizer is unknown or only guessed. When these nitrogen compounds are known and their action on plants as well as the action of the compounds which will be formed from them during their decomposition in the soil, has been determined, then the question of the availability of the nitrogen of organic fertilizers can be understood. Originally it was held that plants were only able to use nitrogen when

it was offered to them in the form of nitrates; this idea, however, was modified when it was discovered that under certain conditions plants used ammonia or ammonium salts without their conversion into nitrates quite as well as they used the nitrates themselves. During the past few years it has been clearly demonstrated that plants not only use nitrogen in the form of nitrates and ammonia but that they can also use nitrogen in the form of complex organic compounds.[1] The action of a number of these nitrogenous compounds has been tested in this laboratory in conjunction with the three fertilizer elements and it has been found that in some cases the nitrogen compounds are not only used as a source of nitrogen for the growing plant, without any change in the compound, but that these compounds were apparently nitrate sparers; that is, the plant used them in preference to the nitrates. Instead, then, of only one kind of nitrogen compound, nitrate, or at most two, nitrate and ammonia, there appears to be a very large number of nitrogenous compounds which have properties of physiological importance to plant growth. The question of the availability of nitrogen compounds can therefore be answered only when the nitrogen compounds contained in the fertilizer can be determined in amount and at the same time classified according to their physiological action on plant growth. It is hardly necessary to state that such a method does not exist at present and that the physiological action of only a part of the total number of nitrogenous compounds present in fertilizers is known.

The physiological action on plants of all of the nitrogenous compounds isolated from base goods has been determined by means of water cultures [2] and the results obtained may be stated briefly, as follows: Both of the purine bases are used by the plant as a source of nitrogen and are beneficial to plant growth; furthermore, the hypoxanthine acts as a nitrate sparer, there being less nitrate used by the plant in the presence of hypoxanthine than when the hypoxanthine is absent. Histidine, arginine, and lysine [3] are all beneficial to plant growth, causing nitrogen increases in the plant, and the two first diamino acids act as nitrate sparers; this may also be true of lysine, although this property of lysine has not been studied. Leucine is also beneficial to plant growth, and tyrosine, in the light of later investigations, is somewhat doubtful in action. Of the other monoamino acids which may be present in base goods, aspartic acid, glutamic acid, and glycocoll have been found to be beneficial. The action of alanine is somewhat doubtful, it apparently being beneficial in low concentrations, and the action of phenylalanine is reported as harmful. Thus we see that six of the seven compounds

[1] Hutchinson and Miller, Centralbl. f. Bakt., 30, 513 (1911); Schreiner and Skinner, Bul. 87, Bureau of Soils, U. S. Dept. Agr., 1912.

[2] Bul. 87, Bureau of Soils.

[3] Unpublished data.

isolated from the base goods are actually available to plants as such and have a beneficial action. Of the monoamino acids, other than the two isolated from base goods, which have been studied in regard to their action on plant growth, three have been found to be beneficial, one doubtful, and one is reported as being harmful.

The high-grade nitrogenous fertilizers, such as dried blood, are considered to have a high availability owing to the fact that the nitrogenous materials when placed in the soil quickly undergo the process of ammonification and nitrification, the nitrogen thus being changed into a form which can be immediately used by the plant. In fact, Lipman [1] has proposed a method for the determination of the availability of the nitrogen of organic fertilizers, depending on the amount of ammonia produced under certain conditions in a given length of time. It is evident from the above consideration that such a method does not tell the whole story, since in the decomposition of protein materials like dried blood intermediate compounds are formed which are undoubtedly in themselves beneficial to plant growth. In order, therefore, to understand the complete action of the nitrogenous materials in the base goods it is necessary to know how the compounds contained in it are acted upon by ammonifying bacteria. Jodidi [2] has shown that the amino acids, and acid amides are quite readily ammonified when placed in the soil, the rate of ammonia formation and the amount of ammonia formed depending apparently upon the chemical structure of the particular compound under consideration. In general, he found that the simpler the chemical structure of the nitrogen compound the more quickly and readily it was ammonified. In the light of these facts it appears that polypeptids, peptones, proteoses, and proteins would be ammonified still more slowly than the amino acids since their structure is increasingly more complex.

Hartwell and Pember [3] in their study on the availability of the nitrogen of base goods, by means of plant tests found that it had apparently as high an availability as dried blood; the water soluble nitrogen having even a higher availability. From the nature and amounts of the compounds present in the base goods this might be predicted. In the case of the dried blood, the nitrogen is practically all in the form of complex protein material which must be broken down into simpler compounds by bacterial action, with the formation of ammonia and other nitrogenous compounds, some or all of which may be of physiological importance to plants. With the base goods the case is a little different, the greater part of the nitrogen is at once available for plant use, and at the same time these available compounds may be changed more easily and quickly by the bacteria

[1] Bul. 246, New Jersey Expt. Sta., 1912.
[2] Research Bul. No. 9, Iowa Expt. Sta.
[3] Loc. cit.

of the soil into ammonia and nitrate, which in turn are used by the plant. The soluble nitrogen of base goods should therefore be in a more readily available form than the nitrogen of dried blood or other nitrogenous fertilizers which are entirely of a protein nature.

## THE CHEMICAL PRINCIPLES UNDERLYING THE UTILIZATION OF NITROGENOUS TRADE WASTES.

In these days of conservation and scientific management more and more attention is being paid to the trade wastes from the various industries and to the municipal scrap heaps. Things which were formerly thrown away are now often made to pay for the entire cost of production. After the resources of the chemist and inventor have failed in finding any other use for some industrial waste, if it be of a nitrogenous nature, the fertilizer industry is turned to as a last resort. Here, however, all is not plain sailing since many of these nitrogenous substances are of such a nature that the nitrogen is said to be "unavailable" for plant use, that is, the substance is of such a nature that it is not readily decomposed by the natural agencies at work in the soil, so that for the purpose of plant nutrition the nitrogen of such substances is worthless or of little value. In order to render available this type of nitrogenous material many different kinds of treatment have been suggested, and the patent literature abounds in inventions of this sort.

It has already been stated that in order that the plant may make use of the nitrogen of even high-grade organic fertilizers, it is necessary for the proteins therein to be at least partially decomposable by the biological and biochemical agencies of the soil. The low-grade organic nitrogenous fertilizers resist decomposition by these biological and biochemical soil agencies, and their nitrogen is therefore considered to be less available for plant use. The guiding idea behind the processes proposed for the treatment of trade wastes, which will not decompose easily in the soil as such, is to change the nitrogen compounds contained in them in such a way that ammonia is formed and that their decay in the soil is more rapid.

Much of the nitrogenous materials in trade wastes is of a protein nature, since the products from which these wastes are derived are either of animal or vegetable origin. Such is the case with the wastes used in the manufacture of base goods. It has been shown that by the process used in the case of this fertilizer the nonavailable nitrogenous materials have been made highly available, not only because the nitrogen compounds can be ammonified quickly in the soil, but also because these compounds are directly utilizable by plants. This change in the nature of the nitrogen compounds has been brought about by the partial hydrolysis of the proteins contained in the various trade wastes used in the manufacture of the fertilizer. When proteins

decompose through natural conditions, be they in the soil or out of it, a certain amount of hydrolysis of the proteins takes place and if the decomposition is allowed to proceed long enough under proper conditions complete hydrolysis will result.

The principle involved in making the nitrogenous material in the soil available and in increasing the availability of low-grade nitrogenous materials by factory treatment is therefore the same. In other words, the general chemical principle to be applied in making available the nitrogen of low-grade fertilizers, trade wastes, etc., is that of complete or partial hydrolysis by any suitable means of the proteins contained in the wastes. Partial hydrolysis of proteins may be accomplished by means of heat, boiling, steaming, heating under pressure, and both partial and complete hydrolysis may be obtained by treating with strong acids or alkalis, either in the cold for a long time or heating to a high temperature, the extent of hydrolysis depending on the several conditions. In a number of processes already in use various of these treatments are practiced, resulting in different degrees of hydrolysis of the original proteins. While the availability of the nitrogen of a fertilizer depends on the substances in which the nitrogen is contained, it also depends on the extent of hydrolysis of the proteins used in the manufacture. It may be stated that in general the more extended and final the hydrolysis the more available the nitrogen of the compounds formed, since as has been shown, the final products of hydrolysis are utilized by the plant as such and are at the same time more readily changed into ammonia by bacteria, etc., than are the intermediate compounds produced by partial hydrolysis.

## SUMMARY.

The base goods used as a type of processed fertilizers is an organic nitrogenous fertilizer which contains acid phosphate. This product is produced by the action of sulphuric acid on certain trade wastes; the heat is generated by the interaction of the acid with the organic wastes and rock phosphate in the course of the manufacture of acid phosphate. It is here shown that the hydrolysis of the protein is almost complete, the nitrogenous compounds in the finished fertilizer being principally the products of primary protein decomposition, together with a small amount of a proteoselike compound which has persisted.

From the sample of base goods were isolated the following nitrogenous compounds, two purine bases, guanine and hypoxanthine; the three diamino acids, arginine, histidine, and lysine; and two monoamino acids, leucine and tyrosine. A proteoselike compound was also obtained and its general nature established.

By means of the Van Slyke method the approximate proportions of the different forms of nitrogen contained in the fertilizer were

estimated, and the extent of the hydrolysis of the original proteins was determined. It was also shown by this method that the proteose-like compound was composed of acid amide radicals, diamino acid radicals, especially lysine, and monoamino acid radicals, particularly the monoamino acids which contain non-amino nitrogen.

The question of the availability of nitrogen is discussed and from a consideration of the amount and the physiological action on plants of the different forms of nitrogen present in the fertilizer it is concluded that the water soluble nitrogen of this fertilizer should have an availability equal to or greater than the nitrogen of dried blood, or other high-grade fertilizers. These results are in accord with the results obtained by the plant method of determining availability.

The general chemical principle which underlies the method for rendering available the nitrogen contained in most trade wastes, which are to be used as fertilizing materials, is shown to be either partial or complete hydrolysis of the protein of the wastes by any suitable means.

The more complete the hydrolysis the more available the nitrogen in the fertilizer becomes, since the products of complete hydrolysis of proteins are not only utilized by the plants themselves as nutrients but they are more easily ammonified when placed in the soil than are the more complex compounds, such as peptones, proteoses, and the proteins themselves.

This investigation aims only at an explanation and exposition of the general chemical principles involved in the treatment of trade wastes and other organic material to render the nitrogen contained therein more available for agricultural purposes. It does not aim to present the research methods here employed as general methods for analyzing such fertilizers, nor can the quantitative figures obtained be expected to apply to all products of similar manufacture, for the reason that the different kinds of nitrogen compounds will necessarily show different proportions according to the nature of the materials which enter into the mixture.

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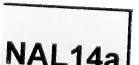
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